Avian Lens Spectrin: Subunit Composition Compared with Erythrocyte and Brain Spectrin

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ABSTRACT Chicken lens spectrin is composed predominantly of equimolar amounts of two polypeptides with solubility properties similar, but not identical, to erythrocyte spectrin. The larger polypeptide, M_r 240,000 (lens α-spectrin), co-migrates with erythrocyte and brain α-spectrin on one- and two-dimensional SDS polyacrylamide gels and cross-reacts with antibodies specific for chicken erythrocyte α-spectrin; the smaller polypeptide, M_r 235,000 (lens γ-spectrin), co-migrates with brain γ-spectrin and does not cross-react with either the α-spectrin antibodies or antibodies specific for chicken erythrocyte β-spectrin. Minor amounts of polypeptides antigenically related to erythrocyte β-spectrin with a greater electrophoretic mobility than lens γ-spectrin are also detected in lens. The equimolar ratio of lens α- and γ-spectrin is invariantly maintained during the extraction of lens plasma membranes under different conditions, or after immunoprecipitation of whole extracts of lens with erythrocyte α-spectrin antibodies. Two-dimensional peptide mapping reveals that whereas α-spectrins from chicken erythrocytes, brain, and lens are highly homologous, the γ-spectrins, although related, have some cell-type-specific peptides and are substantially different from erythrocyte β-spectrin. Thus, the expression of cell-type-specific γ- and β-spectrins may be the basis for the assembly of a spectrin-plasma membrane complex whose molecular composition is tailored to the functional requirements of the particular cell-type.

The cytoskeleton of vertebrate lens cells is composed of microtubules, actin filaments, and vimentin-type intermediate filaments (5, 20, 21, 33, 34, 36). Recent studies have indicated that the lens cytoskeleton may play an important role in lens differentiation. Terminal differentiation of lens fiber cells occurs by a gradual process of cellular elongation as cells are displaced laterally from the periphery of the lens towards the center (reviewed in references 1, 4, 17). During this process there is an increase in the ratio of F-actin to G-actin suggesting that cellular elongation is accompanied by an increase in actin filament assembly (34). An active role of actin filaments in cellular elongation is supported also by the results of studies on lens differentiation in vitro that have shown that the disruption of actin filaments with cytochalasin B or D has an inhibitory effect on this process (28). Although the basis of cellular elongation is not fully understood, it has been suggested that it may involve, at least in part, the interaction between actin filaments and the plasma membrane (34, 40). In lens fiber cells, actin filaments have been observed frequently in the subcortical region where they appear to be attached to the plasma membrane (1, 33, 35, 36).

We have begun to investigate the polypeptide composition of lens cell plasma membranes in order to identify polypeptides involved in this interaction. Initial studies from this laboratory have shown that a polypeptide serologically related to the α-subunit of erythrocyte spectrin is expressed in lens cells and is localized at the cytoplasmic surface of the plasma membrane (37; see also reference 24). In the erythrocyte, spectrin is composed of two nonidentical polypeptides termed α-spectrin (M_r 240,000) and β-spectrin (M_r 220,000) and is found on the inner surface of the plasma membrane where, in association with actin and several other polypeptides, it forms a dense protein meshwork thought to be involved in the maintenance of cell shape and the structural integrity of the plasma membrane (reviewed in reference 6). Recent results have shown that erythroid spectrin and spectrin analogues found in several nonerythroid cell-types have many biochemical and functional properties in common; both form tetramers (2, 12, 38), bind actin (2, 7, 8, 11, 12) and calmodulin (13, 19, 31), and are associated with the cytoplasmic surface of the plasma membrane (2, 8, 24, 25, 29, 30, 37; for reviews see references 6, 23).

In this study we have partially characterized chicken lens spectrin and show that it is composed predominantly of a closely spaced polypeptide doublet; a M_r 240,000 polypeptide (lens α-spectrin), serologically related to chicken erythrocyte α-spectrin, and a M_r 235,000 polypeptide (lens γ-spectrin) that does not react with antibodies directed against either

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chicken erythrocyte α- or β-spectrin. In addition, small amounts of polypeptides related to erythrocyte β-spectrin are detected. The polypeptide composition and electrophoretic mobility of lens spectrin is similar to fodrin (also called brain spectrin), a polypeptide doublet found previously in the cells of nervous tissue (2, 25) (referred to here as brain α- and γ-spectrin). Two-dimensional peptide mapping reveals that α-spectrin from chicken lens and brain are highly homologous, and show high homology also to chicken erythrocyte α-spectrin. The lens and brain γ-spectrins, although related, appear to have a few different peptides; both are different from erythrocyte β-spectrin. This supports the hypothesis that in chickens α-spectrin is highly conserved among different cell types, while polypeptides with which it forms a stoichiometric complex are polymorphic and exhibit cell-type-specific patterns of expression (13, 23, 29).

MATERIALS AND METHODS

Materials: Adult chicken lenses were obtained from a local slaughterhouse, decapsulated, and stored in liquid nitrogen. Chicken erythrocyte membranes were prepared as described (15). Antibodies against electrophoretically purified chicken erythrocyte α-spectrin and β-spectrin have been described (29, 37). The IgG fractions were partially purified by precipitation with ammonium sulfate at 50% saturation at 4°C.

Immunoprecipitation: Immunoprecipitation was performed essentially according to the method of Lingappa et al. (26). Briefly, cells were dissolved in 10 mM Tris-HCl, pH7.4, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1% SDS by boiling for 2 min. The sample was then diluted 10-fold with 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 130 mM NaCl, 0.1% SDS, 1% (vol/vol) Nonident P-40 (immunoprecipitation buffer). To reduce nonspecific binding, samples were precleared by incubation with 5 μl of the preimmune rabbit serum and 50 μl of a 10% (vol/vol) suspension of formalin-fixed *Staphylococcus aureus* (Pansorbin; Calbiochem-Behring Corp., La Jolla, CA) for 2 h. The *S. aureus* was pelleted by centrifugation at 12,000 *g* for 3 min in an Eppendorf microcentrifuge and the supernatant was removed for specific immunoprecipitation with either α-spectrin or β-spectrin antibodies (1.5 μl). The samples were incubated overnight at 4°C. 50 μl *S. aureus* were added for 2 h to bind immune complexes and then centrifuged as above and washed three times in immunoprecipitation buffer. The final pellet was resuspended in 80 μl of SDS-sample buffer (see below), boiled for 2 min, and centrifuged; the polypeptide composition of the supernatant was analyzed by 10% SDS PAGE (see below).

PAGE: One-dimensional SDS PAGE was based on the system of Laemmli (22) as modified and described previously (18). Separating gels contained either 12.5% acrylamide or 10% acrylamide as detailed in the figure legends. The samples were solubilized in 1% (wt/vol) SDS, 125 mM Tris-HCl, pH 6.8, 10% (vol/vol) glycerol, 1 mM EDTA, 1% 2-mercaptoethanol, 0.004% bromophenol blue (SDS-sample buffer). Immunoautoradiography (9) was performed as described previously (14). Gels were exposed on Kodak X-Omat AR film with or without intensifying screens for 2–4 d at −80°C (for details see figure legends).

Solubility Properties of Lens Spectrin: Decapsulated lenses were thawed in ice-cold buffer A (9 mM Tris, 3 mM EDTA, pH 7.4 at 0°C) containing 0.1 mM dithiothreitol and 0.1 mM PMSF and homogenized in a glass homogenizer with 10 strokes of a Teflon pestle. All subsequent operations were performed at 0–4°C unless noted otherwise. Insoluble material was pelleted at 30,000 *g* for 10 min and then resuspended as above and pelleted again. The insoluble material was divided into six equal parts, which were incubated for 1 h on ice in the following solutions: (*a*) buffer A; (*b*) 1% (vol/vol) buffer A in water (incubated at 37°C for the final 30 min); (*c*) buffer A containing 1% (wt/vol) Triton X-100, 0.1 mM dithiothreitol and either 0.1 M or 1 M KCl; and (*d*) buffer A containing 2 mM diamide (two aliquots). Subsequently, the diamide-treated material was extracted for 1 h on ice with buffer A containing 6 M urea and 0.1 M NaCl, in the presence or absence of 20 mM dithiothreitol. At the end of the incubation period each sample was centrifuged at 30,000 *g* for 10 min. The resulting supernatants were dialyzed against water, lyophilized and boiled in SDS-sample buffer; Triton X-100 that remained after dialysis was extracted from the lyophilized sample with 100% ethanol. Residues were boiled directly in SDS-sample buffer; those containing KCl and Triton X-100 were first rinsed with buffer A.

Two-dimensional Peptide Mapping: Chicken erythrocyte β'- and β-spectrin and brain and lens γ-spectrin were purified by SDS PAGE and excised from the gels. Each gel slice was dialyzed extensively against 10% (vol/vol) ethanol and lyophilized. The proteins were then iodinated (carrier-free Na-^{125}I, 17.4 Ci/mg; New England Nuclear, Boston, MA) in the gel slices according to the procedure of Elder et al. (10), followed by washing with 8–10 changes of 10% (vol/vol) methanol, 2 mM tyrosine over a 2-d period. Alternatively, for erythrocyte, brain and lens α-spectrin, the polypeptides were iodinated in a solution of 1% (wt/vol) SDS, 100 mM Na·PO_4 buffer, pH 7.4, before PAGE. Similar peptide maps were obtained if the α-spectrins were excised from SDS gels and then radioiodinated as described above (data not shown). The gel slices were lyophilized and then incubated initially in 500 μl of α-chymotrypsin (Worthington Biochemical Corp., Freehold, NJ; 50 μg/ml) in 200 mM NH_4HCO_3 for 12 h at 37°C, followed by the addition of a further 500-μl aliquot of freshly prepared α-chymotrypsin (as above) and incubation for 24 h at 37°C. Aliquots (10–30 μl) were lyophilized and then separated on cellulose-coated Eastman chromatography sheets (13255) by high voltage electrophoresis in the first dimension and ascending chromatography in the second dimension essentially according to the method of Elder et al. (10). The chromatograms were exposed on Kodak X-Omat AR film with intensifying screens for 12–24 h at −80°C.

RESULTS

Immunological Characterization of Lens Spectrin

The identification of lens polypeptides antigenically related to chicken erythrocyte spectrin was determined by immunoautoradiography using highly specific antibodies raised against the α- and β-subunits of erythrocyte spectrin (Fig. 1).

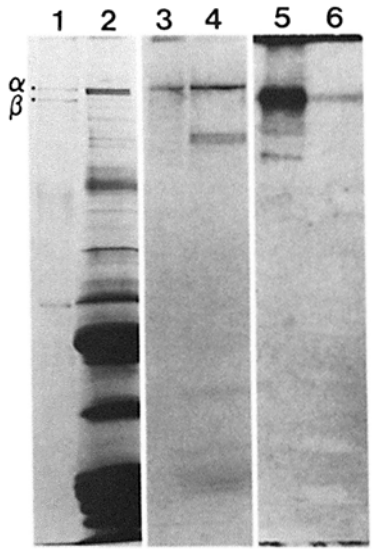

FIGURE 1 Characterization of chicken erythrocyte and lens spectrin by immunoautoradiography with antibodies specific for the α- and β-subunits of chicken erythrocyte spectrin. Lane *1*, Coomassie Blue-stained SDS polyacrylamide gel of chicken erythrocyte membranes and the corresponding autoradiograms following incubation of duplicate gels with α-spectrin antibodies (lane *3*) and β-spectrin antibodies (lane *5*). Lane *2*, Coomassie Blue-stained gel of whole lens and the corresponding autoradiograms following incubation of duplicate gels with α-spectrin antibodies (lane *4*) and β-spectrin antibodies (lane *6*). The autoradiograms in lanes *3* and *4* were exposed for 5 d without intensifying screens, those in lanes *5* and *6* were exposed for 4 d with intensifying screens. In lane *4*, the two lower molecular weight polypeptides of lens α-spectrin probably represent proteolytic fragments similar to those shown previously for erythrocyte α-spectrin (see Fig. 3, reference 37). Similarly, the lower molecular weight polypeptides below erythrocyte β-spectrin (lane *5*) also represent proteolytic fragments (see reference 29). The presence of two β-spectrin variants in chicken erythrocytes is not clear in lane *5* due to the long exposure of the autoradiogram required to detect cross-reacting polypeptides in lens.

A prominent high molecular weight polypeptide with a relative electrophoretic mobility similar to that of erythrocyte α-spectrin (M_r 240,000), reacts strongly with the α-spectrin antibodies (reference 37; and Fig. 1, lanes *1–4*). Additionally, minor quantities of two polypeptides (M_r 230,000 and 225,000) antigenically related to the two variants of erythrocyte β-spectrin are detected (Fig. 1, lanes *5* and *6*).

Immunoprecipitation of SDS-solubilized lens polypeptides with erythrocyte α-spectrin antibodies reveals that a closely spaced polypeptide doublet is specifically immunoprecipitated (Fig. 2, Lane *1*). The upper component corresponds to lens α-spectrin as determined by immunoautoradiography (see above). However, as shown in Fig. 1, the lower component (M_r 235,000) does not cross-react with either erythrocyte α- or β-spectrin antibodies and, furthermore, is not immunoprecipitated with either preimmune serum or β-spectrin antibodies (Fig. 2, lanes *4* and *5*). The molar ratio of the components of the polypeptide doublet is approximately 1:1 following immunoprecipitation with α-spectrin antibodies and is similar to that of the doublet in whole lens as assayed by one-dimensional SDS PAGE (compare Fig. 1, lanes *1* and *2*). The co-immunoprecipitation of equimolar amounts of SDS-solubilized α- and γ-spectrin is probably the result of reassociation of the spectrin subunits during the course of the incubation with the α-spectrin antibodies. A similar result has been obtained also during the immunoprecipitation of chicken erythrocyte spectrin subunits with either α- or β-spectrin antibodies (3). Furthermore, mammalian α- and β-spectrin have been shown previously to be capable of reassociation after denaturation (16, 27). The equimolar ratio of α:γ-spectrin is maintained during extraction of whole lens and lens membranes with various buffers (see below), thus supporting the hypothesis that the M_r 235,000 polypeptide interacts with lens α-spectrin on an equimolar basis, and is likely, therefore, to be a member of the spectrin family. Since this polypeptide is not antigenically related to either erythrocyte α- or β-spectrin, it is referred to as lens γ-spectrin.

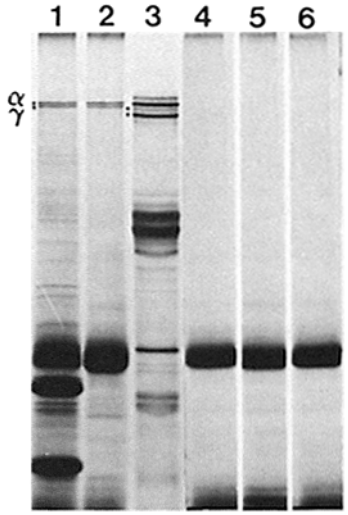

FIGURE 2 Comparison of chicken lens and brain spectrin by immunoprecipitation. Chicken lens and cerebellum polypeptides were solubilized and immunoprecipitated with α- and β-spectrin antibodies as described in Materials and Methods. Coomassie Blue-stained SDS gels of α-spectrin immunoprecipitates of lens (lane *1*) and cerebellum polypeptides (lane *2*), chicken erythrocyte plasma membrane proteins as molecular weight markers (dots represent position of β′- and β-spectrin) (lane *3*), preimmune serum immunoprecipitate of lens polypeptides (lane *4*), β-spectrin immunoprecipitates of lens (lane *5*), and brain polypeptides (lane 6). The identity of the low molecular weight polypeptides in the α-spectrin immunoprecipitates of lens (lane *1*) is unknown.

Erythrocyte β-spectrin antibodies do not appear to immunoprecipitate any polypeptide detectable by Coomassie Blue staining from extracts of whole lens (Fig. 2, lane *5*). However, we have shown previously that immunoautoradiography with β-spectrin antibodies of similar immunoprecipitates demonstrates the presence in lens of small quantities of two polypeptides with electrophoretic mobilities similar to those of erythrocyte β- and β′-spectrin (29), in accordance with the immunoautoradiograms of whole lens extracts (Fig. 1, lane *6*).

Comparison of the Subunit Composition of Lens Spectrin with That of Brain and Erythrocyte Spectrin

The polypeptide composition of lens spectrin appears to be similar to that of brain spectrin (also termed fodrin [25]). Brain spectrin is a high molecular weight polypeptide doublet specifically immunoprecipitated from extracts of whole chicken brain with chicken erythrocyte α-spectrin antibodies, and has a relative electrophoretic mobility identical to that of lens spectrin (Fig. 2, lanes *1* and *2*). Two-dimensional isoelectric focusing SDS PAGE shows that lens and brain α-spectrin have isoelectric points and electrophoretic mobilities similar to chicken erythrocyte α-spectrin. Lens and brain γ-spectrin do not focus discretely in the system used in these studies, occasionally appearing as a streak of unfocused protein leading from the origin of the isoelectric focusing gel (data not shown). Under these electrophoretic conditions, chicken erythrocyte β-spectrin also does not focus discretely (15, 37).

To further characterize the relationship between chicken erythrocyte, lens and brain spectrin, we compared each of the subunits using two-dimensional peptide mapping (Fig. 3). There is a high degree of homology between the α-spectrins of avian erythrocyte, lens and brain (Fig. 3, *a*, *d*, and *f*) indicating that the primary structures of avian erythroid and nonerythroid α-spectrins are very similar if not identical. A comparison of the γ-spectrin peptide maps (Fig. 3, *e* and *g*) indicates a considerable degree of homology between lens and brain, but that there are some cell-type-specific differences. We cannot exclude, however, the possibility that these differences are the result of a minor contamination of the samples by other proteins. This possibility appears to be unlikely since these differences were reproducible from sample to sample. The lens and brain γ-spectrins show significant differences from erythrocyte β′- and β-spectrin (Fig. 3, *b* and *c*).

Solubility Properties of Lens Spectrin

To investigate the stability of the association of lens α- and γ-spectrin with each other and with the lens plasma membrane, we extracted lenses under a variety of ionic conditions (Fig. 4). The extracts and residues from equivalent amounts of starting material (except in Fig. 4, lane *2*) were separated by one-dimensional SDS PAGE. Extraction of lenses with 9 mM Tris, 3 mM EDTA, pH 7.4 (buffer A), containing 0.1 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride results in the solubilization of little spectrin (Fig. 4, lane *2*). The majority of the polypeptides released represent crystallins, which are soluble in water or buffers of low ionic strength.

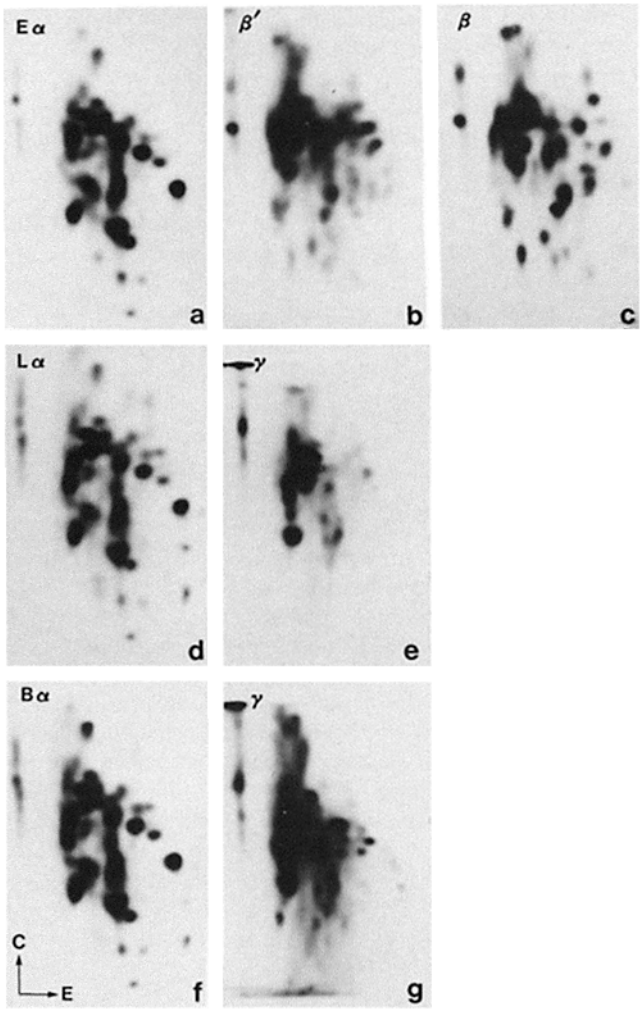

FIGURE 3 Comparison of the two-dimensional chymotrypsin iodopeptide maps of the subunits of chicken erythrocyte, lens, and brain spectrin. Electrophoretically purified and iodinated spectrin subunits were digested with α-chymotrypsin and the resulting iodopeptides separated by high voltage electrophoresis in the first dimension (*E*) and ascending chromatography in the second dimension (*C*). Resulting autoradiograms of the following: chicken erythrocyte α- (*a*), β′- (*b*), and β-spectrin (*c*); lens α- (*d*) and γ-spectrin (*e*); brain α- (*f*) and γ-spectrin (*g*). The peptide maps of lens γ-spectrin (*e*) and brain γ-spectrin (*g*) are slightly overexposed to show the minor differences. Peptides in the overexposed areas have almost complete homology in the two tissues (not shown).

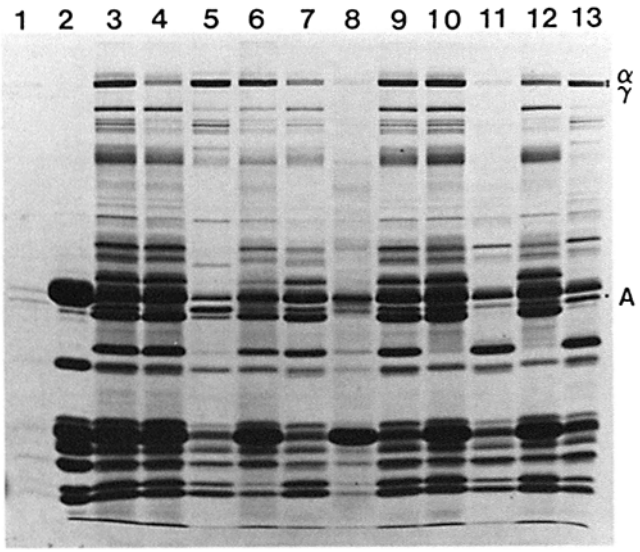

FIGURE 4 Analysis of the solubility properties of chicken lens by 12.5% SDS PAGE of various lens extracts and residues. Each lane represents an equivalent amount of starting material (about one-third of a decapsulated lens), except for lane *2* that represents 2% of this amount. Lane *1* and *2*: third and first extract, respectively, and lane *3* the third residue of lens fibers homogenized in a hypotonic Tris/EDTA solution. Lane *3* represents the insoluble lens fiber material for the following treatments. Lanes *4* and *5*: residue and extract of lens fibers incubated at 37°C in 0.09 mM Tris, 0.03 mM EDTA, pH 6.8. Lens fibers oxidized with diamide are shown in lanes 6–9; lanes *6* and *7* are the corresponding residue and extract obtained after a subsequent treatment with 6 M urea under non-reducing conditions; lanes *8* and *9* are the corresponding residue and extract of a subsequent treatment with 6 M urea in the presence of a reducing agent (DTT). Lanes *10* and *11* are the corresponding residue and extract of lens fibers treated with Triton X-100 in 0.1 M KCl, lanes *12* and *13* are analogous, except 1 M KCl was used. For further details see Materials and Methods, and the text.

Further extraction of the insoluble material for two more cycles with buffer A results in the solubilization of an additional small amount of the spectrin (Fig. 4, lane *1*). Equivalent amounts of insoluble material from the first extraction with buffer A (as in Fig. 4, lane *3*) were used for all subsequent treatments. Further extraction with 0.09 mM Tris, 0.03 mM EDTA, pH 7.4, at 37°C results in the release of the majority of the spectrin together with most of the actin (Fig. 4, lanes *4* and *5*). To analyze the effect of cross-linking on the extractability of spectrin, the insoluble material was treated with 2 mM diamide, a thiol-oxidizer, in buffer A for 1 h at 0°C, and then extracted with 6 M urea in the absence (Fig. 4, lanes *6* and *7*) or presence (Fig. 4, lanes *8* and *9*) of 20 mM dithiothreitol. The results show that pretreatment with diamide causes most the spectrin to remain insoluble in urea (Fig. 4, lane *6*), but that this effect can be almost completely reversed by inclusion in the urea solution of the thiol-reducing agent, dithiothreitol (Fig. 4, lane *8*). During both treatments, most of the actin is solubilized. In the presence of 0.1 M KCl and 1% (wt/vol) Triton X-100, most of the spectrin and actin remain insoluble (Fig. 4, lane *10*). However, by increasing the concentration of KCl to 1 M in the extraction buffer, over half of the spectrin and nearly all of the actin is solubilized (Fig. 4, lane *13*). In general these results are comparable with those obtained recently on the solubility properties of chicken erythrocyte spectrin from erythrocyte plasma membranes (37). However, the degree to which chicken erythrocyte spectrin and lens spectrin can be rendered insoluble with diamide appears to be different. The extraction of diamide-treated chicken erythrocyte membranes with 6 M urea releases little spectrin (37), whereas approximately one-third of lens spectrin is extractable from lens plasma membranes under similar conditions. Finally, it should be noted that the equimolar ratio of lens α-spectrin and γ-spectrin is invariantly maintained in all supernatants and residues following extraction in buffers of low or high ionic strength, in the presence of detergents or reducing agents, and under denaturing conditions.

DISCUSSION

The role of the cytoskeleton in several membrane activities, such as the capping of cell-surface receptors, cell motility and attachment of cells to a substratum, involves the association of one or more of its components with the plasma membrane

(39). In lens fiber cells, actin filaments have been shown by a number of criteria to be closely associated with the plasma membrane (1, 33, 35, 36). The disruption of the actin filament system inhibits lens differentiation in vitro as shown by the cessation of cellular elongation (28). These results demonstrate the importance of the interaction of actin filaments with the plasma membrane in lens differentiation, although little is known about the molecular mechanism involved. Recently, however, it has been shown that a polypeptide antigenically related to the α-subunit of the actin-binding protein spectrin is expressed in lens cells and is associated with the plasma membrane (24, 37) indicating that this protein may be involved in linking actin to the membrane. In the present study, we extended these observations to show that lens spectrin is composed predominantly of equimolar amounts of two polypeptides, lens α- and γ-spectrin. Lens α-spectrin (M_r 240,000) reacts with antibodies to erythrocyte α-spectrin, as shown previously (37), while lens γ-spectrin (M_r 235,000) does not bind either erythrocyte α- or β-spectrin antibodies. Minor amounts of polypeptides antigenically related to erythrocyte β-spectrin are also detected. The fact that the lens is avascular and that only decapsulated lenses free of muscle and connective tissue were used in this and our previous study (29), demonstrates unequivocally that lens cells specifically express these β-spectrin-related polypeptides.

In view of the emerging evidence that erythroid spectrin and nonerythroid spectrin analogues have many biochemical and structural properties in common (see introduction), the function of spectrin in lens may be similar to that in erythrocytes. It is known, for example, that actin filaments and intermediate filaments (1, 33, 35, 36) and spectrin (37) are found in close association with or are bound to the lens plasma membrane. These elements of the cytoskeleton may, therefore, be involved in the maintenance of cell shape and the structural integrity of the plasma membrane; these are parameters thought to be important in visual accommodation which is achieved by deformation of the lens (21). During cellular elongation, the spectrin-actin complex may act also as a nucleating site for G-actin polymerization, resulting in the relative increase in the ratio of F- to G-actin observed in lens differentiation (34). This is supported by recent results that show that isolated spectrin-containing oligomers from human erythrocytes nucleate G-actin polymerization (32).

The $\alpha\gamma$-spectrin phenotype expressed in adult chicken lens appears to be similar in subunit composition to that expressed in several other nonerythroid cells such as lymphocytes (30), fibroblasts (8), and brain cells (2, 25). The two-dimensional chymotryptic peptide maps of lens and brain α-spectrin revealed that these polypeptides have highly homologous if not identical primary structures equivalent to that of chicken erythrocyte α-spectrin. Similarly, Glenney et al. (13) have shown that α-spectrin from chicken intestinal terminal web (TW240 in their nomenclature) has a one-dimensional peptide map identical to that of chicken erythrocyte α-spectrin. In mammals, however, the peptide maps of erythroid and nonerythroid (brain) α-spectrin are different (2), although the proteins appear to be antigenically related (2, 8, 24, 38). This difference between erythroid and nonerythroid α-spectrins in mammals but not in chickens may be a reflection of the relative specialization of the anucleate mammalian red cell, in contrast to the chicken erythrocyte that is nucleated and contains an intermediate filament network (15).

Although the α-spectrin expressed in different avian cell-types appears to be the same, the spectrin subunit with which it forms a complex is polymorphic. The two-dimensional peptide maps of the γ-spectrin from lens and brain show that there is considerable homology between the polypeptides, although there are a few peptides which appear to be specific to each. Chicken erythrocytes express two immunologically related polypetides (β- and β'-spectrin) which have very similar, but not identical, peptide maps (29). Neither lens nor brain γ-spectrin show any appreciable homology with erythroid β- or β'-spectrin. Adult cardiac and skeletal muscle cells express polypeptides which, although antigenically related to β-spectrin, have slightly different electrophoretic mobilities in SDS polyacrylamide gels than erythrocyte β- and β'-spectrin (29). Finally, Glenney et al. (13) have shown that the polypeptide (TW260), which interacts with the intestinal terminal web α-spectrin, has a one-dimensional peptide map different from both erythrocyte β-spectrin and brain γ-spectrin and is also antigenically distinct.

What is the biological significance of the variation in the subunit composition of spectrin in different cell types? It is possible that different combinations of subunits may alter the physicochemical properties of the spectrin molecule. This may be reflected, for instance, in differences in the degree of oxidative cross-linking and extractability of avian lens and erythrocyte spectrin, as shown in this study, and in differences in the contour length and sedimentation coefficients of brain, intestinal terminal web, and erythrocyte spectrin tetramers (2, 12, 13). However, it is particularly significant that the variation in spectrin subunit composition is not due to the α-subunit, which is common to several avian cell types examined, but to a variation in the subunit that may be functionally analogous to erythrocyte β-spectrin (see also reference 13), which, in erythrocytes at least, is involved in the linkage of the spectrin-actin complex to the plasma membrane (reviewed in reference 6). By expressing cell-type-specific γ-spectrin or β-spectrin, in association with a common α-spectrin the cell may regulate the dynamics of the interaction between the cytoskeleton and cell-type-specific components of the plasma membrane, which may contribute to membrane differentiation and hence tissue-specific membrane properties.

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